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ORIGINAL ARTICLE

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Unilateral and bilateral hybridization barriers in inter-series crosses of 4x 2EBN *Solanum stoloniferum*, *S. pinnatisectum*, *S. cardiophyllum*, and 2x 2EBN *S. tuberosum* haploids and haploid-species hybrids

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Abstract Wild Mexican potato species are an important untapped source of useful variation for potato improvement. Introgression methods such as 2*n* gametes, chromosome doubling, and crossing with disomic 4x 2 endosperm balance number (EBN) bridge species have been used to overcome post-zygotic endosperm failure according to the EBN hypothesis. Stylar barriers can prevent zygote formation, bilaterally when zygote formation is blocked in both directions of the cross or unilaterally when zygote formation is blocked in self incompatible (SI) × self compatible (SC) crosses. In several Solanaceae species, the S-locus for SI has been implicated in interspecific incompatibility. The objectives of this research were to determine if: (1) disomic 4x 2EBN *Solanum stoloniferum* can be used as a bridge species for introgression of the Mexican 2x 1EBN species *Solanum cardiophyllum* and *Solanum pinnatisectum*, (2) pre- and/or post-zygotic barriers limit hybridization among EBN compatible *Solanum* inter-series crosses, and (3) reproductive barriers act unilaterally or bilaterally. Fruit formation and seed set was recorded for inter-pollinations of *S. stoloniferum*, 4x 2EBN chromosome doubled *S. cardiophyllum* and *S. pinnatisectum*, and 2x 2EBN *S. tuberosum* haploids (HAP) or haploid-species hybrids (H-S). *In vivo* pollen tube growth was analyzed for each cross combination with fluorescence microscopy. Attempts to create bridge hybrids between *S. stoloniferum*, and *S. cardiophyllum* or *S. pinnatisectum* were not successful. Pre- and post-zygotic barriers prevented seed formation in crosses involving *S. cardiophyllum* and *S. pinnatisectum*. Self compatibility in *S. stoloniferum* and *S. pinnatisectum* suggests that the S-locus does not contribute to the stylar barriers observed with these species. Alternatively, the presence of functional and nonfunctional (SC) S-alleles may explain interspecific incompatibility in intra- and inter-ploidy crosses. A non-stylar unilateral incongruity was discovered in H-S/HAP × *S. stoloniferum* crosses, indicating either a post-zygotic barrier, or a pre-zygotic barrier acting at or within the ovary. Furthermore, lack of *S. stoloniferum* pollen rejection may occur through absence of *S. stoloniferum* pollen-active genes needed to initiate pollen rejection, or through competitive interaction in S-locus heterozygous *S. stoloniferum* pollen. Introgression strategies using these species would benefit potato breeding by introducing genetic diversity for several traits simultaneously through co-current introgression.

Keywords Bridge crossing · Endosperm balance number · Gene introgression · Interspecific incompatibility · Incongruity

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Introduction

Substantial diversity exists for traits of economic importance in wild relatives of crop species. Reproductive barriers that promote speciation are important for maintaining this diversity. Consequently, understanding these barriers and developing techniques to overcome them is fundamental to exploiting this variation for crop improvement. This is particularly important for introgression of complex quantitatively inherited traits such as yield, yield stability, and non-race-specific resistance to pests, which can not be introduced into cultivated germplasm through gene cloning and transformation. In wild potato species (*Solanum* section *Petota*) many traits have been discovered that can improve cultivated potato (Hanneman 1989), including resistance to biotic and abiotic stresses and improved traits of economic

importance (Spooner and Bamberg 1994). Wild potato species from Mexico are a particularly valuable and untapped resource of variation. For example, virus resistance and aphid resistance were introgressed from *Solanum etuberosum* (Novy et al. 2002). Individual genotypes of *Solanum pinnatisectum* and *Solanum stoloniferum* that combine multiple useful traits have been identified (Hayes and Thill 2002). This germplasm can be used in a co-current introgression effort, a method that is more efficient than introgressing each trait individually (Hayes and Thill 2002).

S. pinnatisectum and *S. cardiophyllum* are primitive 2x species from Mexico and the southwest United States that are reproductively isolated from 4x cultivated potato and their 2x haploids (Novy and Hanneman 1991; Kuhl et al. 2002; Ramon and Hanneman 2002; Dinu et al. 2005). Several approaches for direct introgression into cultivated potato have been used to overcome reproductive barriers between potato species. These include embryo rescue with and without auxin treatment (Dinu et al. 2005), double pollination, ploidy manipulations, and somatic hybridization (Helgeson et al. 1998; Hanneman 1999). Application of these methods to obtain sexual F_1 progeny in direct crosses with Mexican species has been difficult (Novy and Hanneman 1991; Kuhl et al. 2002; Ramon and Hanneman 2002; Dinu et al. 2005). From research using embryo rescue and double pollination, only a single hypoploid hybrid between 2x *S. tuberosum* and 2x *S. pinnatisectum* has been reported (Ramon and Hanneman 2002). The difficulty in direct introgression was explained by early endosperm failure and absence of embryo formation in direct crosses between these species (Dinu and Thill 2004; Dinu et al. 2005).

Crossing with bridge species can be used when direct crossing to cultivars is not practical. The endosperm balance number (EBN) hypothesis is useful for predicted crossing success in *Solanum* species, and therefore is also useful for selecting species for bridge crossing. The EBN hypothesis predicts successful endosperm formation when a 2 maternal:1 paternal ratio of EBN is met, rather than a 2:1 ratio of ploidy (Johnston et al. 1980). A species EBN value is assigned based on experimental crossing results with known testers (Spooner and Hijimans 2001), and can be independent of ploidy. Because of this, the EBN hypothesis can explain seed formation in a wide range of inter-ploidy hybridizations that are useful as bridge crosses (Hanneman 1999). For example, the 4x 2EBN South American disomic species *Solanum acaule* has been hybridized with 2x 1EBN germplasm (Hermsen and Rammana 1973; Bamberg et al. 1994; Budin and Gavrilenko 1994). In this scheme, 4x 2EBN bridge hybrids are created when the 2x 1EBN parent produces 2*n* gametes or is colchicine doubled prior to crossing with *S. acaule*. The bridge hybrids must produce 2*n* gametes to overcome EBN barriers in subsequent backcrosses to 4x 4EBN cultivated potato. *S. stoloniferum* is a 4x 2EBN Mexican species with disomic inheritance that will cross directly with 2x 2EBN genotypes (Adiwilaga and Brown 1991). Furthermore, the EBN hypothesis predicts hybridization between *S. stoloniferum* and colchicine chromosome doubled 4x 2EBN autotetraploids of 2x 1EBN genotypes or 2x 1EBN genotypes producing 2*n* gametes. This fact makes *S. stoloniferum* a good candidate for bridge crossing with Mexican diploid species. The use of *S. stoloniferum* as a bridge species for introgression of Mexican 1EBN germplasm has not been reported in the literature.

Interspecific incompatibility commonly occurs among *Solanum* species, and can occur as stylar barriers that act bilaterally, or unilaterally in self compatible (SC) × self incompatible (SI) crosses (Hanneman 1999). Consequently, crosses may fail despite efforts to overcome post-zygotic EBN barriers. The genetics of interspecific incompatibility has been debated by several authors, and in particular to its relation to the S-locus for SI. Diploids in the *Solanaceae* family are commonly SI due to gametophytic SI (GSI), the specificity of which is controlled by multiallelic S-alleles at the S-locus (Cipar et al. 1964; Cruz-Garcia et al. 2003). Glycoproteins with ribonuclease activity, termed S-RNases, control the pistil specificity of GSI in *Solanaceae*, *Scrophulariacaea*, and *Rosaceae* and are thought to have a common evolutionary origin (Igic and Kohn 2001). S-RNase function is required for unilateral incompatibility (UI) in some interspecific crosses (Murfett et al. 1996; Eijlander 1998). The objectives of this research were to determine if: (1) *S. stoloniferum* can be used as a bridge species for introgression of *S. cardiophyllum* and *S. pinnatisectum*, (2) pre- and/or post-zygotic barriers limit hybridization among EBN compatible haploid-species hybrids, *S. tuberosum* haploids, *S. stoloniferum*, *S. cardiophyllum*, and *S. pinnatisectum*, and (3) reproductive barriers act unilaterally or bilaterally.

Materials and methods

Five 4x 2EBN *S. stoloniferum* accessions (three to six genotypes per accession), one autotetraploid 2EBN *S. pinnatisectum* accessions (four genotypes), one autotetraploid 2EBN *S. cardiophyllum* accession [one genotype; plant introduction (PI) numbers shown in Table 1], six haploid-species hybrid (H-S) genotypes, and one *S. tuberosum* haploid (HAP) genotype were used for these experiments. The *S. pinnatisectum* and *S. cardiophyllum* autotetraploid 2EBN genotypes used in these experiments are all derivatives from colchicine chromosome doubling of 2x 1EBN plants (Zlesak and Thill 2001). These genotypes, along with *S. stoloniferum*, are all SC, while all 2x H-S are SI. The H-S were clones ADX1523-1, c159, c213, c307, c380, and HYB17-1. These are selected diploid breeding lines incorporating the species *S. chacoense*, *S. berthaultii*, *S. sparsipilum*, *S. verrucosum*, *S. stenotomum*, *S. microdontum*, *S. phureja*, and *S. tuberosum*. The HAP used was USW-463, which is derived from the cultivar Katahdin. As with nearly all HAP, this genotype is

Table 1 Species name, abbreviation, ploidy, endosperm balance number (EBN), plant introduction (PI) number, taxonomic series, and self compatibility of parents used in interspecific hybridizations

Species	Ploidy, EBN	PI or clone number	Series	Number of genotypes	Compatibility
Solanum pinnatisectum (pnt)	4x, 2EBN[a]	230489	*Pinnatisecta*	4	Self-compatible
Solanum cardiophyllum (cph)	4x, 2EBN[a]	283062	*Pinnatisecta*	1	Self-compatible
Solanum stoloniferum (sto)	4x, 2EBN	161178	*Longipedicellata*	3	Self-compatible
S. stoloniferum (sto)	4x, 2EBN	195166	*Longipedicellata*	3	Self-compatible
S. stoloniferum (sto)	4x, 2EBN	239410	*Longipedicellata*	6	Self-compatible
S. stoloniferum (sto)	4x, 2EBN	230490	*Longipedicellata*	3	Self-compatible
S. stoloniferum (sto)	4x, 2EBN	161158	*Longipedicellata*	3	Self-compatible
Haploid-species (H-S)	2x, 2EBN	ADX1523-1	*Tuberosa*	1	Self-incompatible
Haploid-species (H-S)	2x, 2EBN	c380	*Tuberosa*	1	Self-incompatible
Haploid-species (H-S)	2x, 2EBN	c159	*Tuberosa*	1	Self-incompatible
Haploid-species (H-S)	2x, 2EBN	c213	*Tuberosa*	1	Self-incompatible
Haploid-species (H-S)	2x, 2EBN	c307	*Tuberosa*	1	Self-incompatible
Haploid-species (H-S)	2x, 2EBN	HYB17-1	*Tuberosa*	1	Self-incompatible
Solanum tuberosum haploid (HAP)	2x, 2EBN	USW463	*Tuberosa*	1	Self-incompatible[b]
Total				30	

[a]Selected colchicine doubled autotetraploid derivative from 2x 1EBN SI genotype
[b]Genotype is male-sterile, presumed SI

male-sterile and was used only as a female. Rare male-fertile HAP of 4x cultivated potato do occur, and are SI (Olsder and Hermsen 1976; Hermsen 1978; Hermsen et al. 1978). Therefore, USW-463 is also likely SI.

Plants were greenhouse grown in 25 cm pots under an artificial 16 h photoperiod. Three to five flowers per inflorescence were emasculated, and receptive stigmas pollinated in the morning with fresh pollen. The number of pollinations per inflorescence was recorded. After more than 5 weeks post pollination, the number of fruit and seeds per fruit were recorded. The percentage fruit set was calculated, and 95% confidence intervals were estimated without continuity correction.

In vivo pollen tube growth was analyzed by collecting pistils 48 h after pollination and incubating the pistils in FAA (1 part formalin:8 parts 80% ethanol:1 part glacial acetic acid) for 24 h. Pistils were then placed in 70% ethanol and stored at 4°C. Prior to evaluation, pistils were washed in tap water, incubated overnight in 8 *N* NaOH, rinsed in tap water for 1 h, and stained in aniline blue (0.5% in 0.1 *N* K_2PO_4). The style alone or the style with one-quarter of the ovary top was gently squashed in aniline blue solution with a cover slip. At least five slides per cross combination were evaluated. Pollen tubes were observed with fluorescence microscopy. A 100 W mercury lamp epi-fluorescence under UV light was used either with a G365 excitation filter, LP520 barrier filter and a SPOT RT Color camera model 2.2.0 digital camera, or using a 330–380 excitation filter and 420 barrier filter with digital images captured using a CoolCam CCD camera (Diagnostic Instruments, Sterling Heights, Mich.). Images were stored and analyzed using Image Pro Plus 4.5 software (Media Cybernetics, Silver Springs, Md.) or ImageJ 1.31 v software (National Institutes of Health; http://rsb.info.nih.gov).

Results

Large differences were observed for fruit formation and seed set for 11 inter- and intra-specific crosses (Table 2). Control crosses, *S. stoloniferum* × *S. stoloniferum*,

Table 2 Fruit and seed formation from nine crosses of *S. stoloniferum* (*sto*), *S. cardiophyllum* (*cph*), *S. pinnatisectum* (*pnt*), haploid-species hybrids (*H-S*), and *S. tuberosum* haploids (*HAP*)

Cross	Pollinations	Mature fruit formation		Plump seeds/fruit
		Percent fruit (number observed)	95% confidence interval	
sto (4x 2EBN) × cph (4x 2EBN)	105	0 (0)[a]	0–2	–
sto (4x 2EBN) × pnt (4x 2EBN)	71	0 (0)	0–5	–
sto (4x 2EBN) × H-S (2x 2EBN)	142	66 (94)	58–73	52
sto (4x 2EBN) × sto (4x 2EBN)	176	64 (113)	57–71	86
H-S/HAP (2x 2EBN) × sto (4x 2EBN)	69	0 (0)	0–5	–
H-S/HAP (2x 2EBN) × pnt (4x 2EBN)	49	0 (0)	0–7	0
H-S/HAP (2x 2EBN) × H-S (2x 2EBN)	228	20 (46)	15–26	182
pnt(4x 2EBN) × sto (4x 2EBN)	148	3 (5)	1–8	0[b]
pnt(4x 2EBN) × cph (4x 2EBN)	44	18 (8)	10–32	6
pnt(4x 2EBN) × H-S (2x 2EBN)	235	4 (10)	2–8	0
pnt(4x 2EBN) × pnt (4x 2EBN)	23	22 (5)	10–14	33
Total	791	34 (271)	0–71	88

[a]Fruit aborted 7–10 days after pollination
[b]Many aborted flat seeds

S. pinnatisectum × *S. pinnatisectum*, H-S/HAP × H-S, and *S. pinnatisectum* × *S. cardiophyllum* formed fruit and seeds; indicating that the parents used had sufficient fertility for sexual reproduction. However, these parents did not have equally effective fertility. Fruit formation was significantly lower for *S. pinnatisectum* × *S. pinnatisectum* ($P \leq 0.01$), *S. pinnatisectum* × *S. cardiophyllum* ($P \leq 0.01$), and H-S/HAP × H-S ($P \leq 0.01$) when compared to *S. stoloniferum* × *S. stoloniferum*. While 182 seeds per fruit were observed in H-S/HAP × H-S crosses, only 33 and 6 seeds per fruit were observed in *S. pinnatisectum* × *S. pinnatisectum* and *S. pinnatisectum* × *S. cardiophyllum* crosses, respectively.

Wide variation was observed for fruit formation and seeds per fruit in interspecific crosses using *S. stoloniferum* as the female parent. Fruit formation in *S. stoloniferum* × H-S was significantly greater than in any other interspecific cross as well as control crosses ($P \leq 0.01$), and was not statistically different from *S. stoloniferum* × *S. stoloniferum* ($P \leq 0.01$) (Table 2). In addition, large numbers of seeds per fruit were observed in *S. stoloniferum* × H-S. In contrast, no fruit formation was observed in the reciprocal cross H-S/HAP × *S. stoloniferum*. For *S. stoloniferum* × *S. cardiophyllum* crosses, no mature fruit was recovered; fruit formation was initiated but then aborted 7–10 days after pollination and fruit never reached diameters greater than 1 cm, and did not contain seeds. No fruit was observed for *S. stoloniferum* × *S. pinnatisectum*, or in H-S/HAP × *S. pinnatisectum*. In the reciprocal crosses *S. pinnatisectum* × *S. stoloniferum* and *S. pinnatisectum* × H-S, 3–4 % fruit formation was observed. Fruit from *S. pinnatisectum* × *S. stoloniferum* crosses contained numerous seeds; however, they were flat, aborted, and non-viable.

Variability in pollen-pistil interaction among crosses was observed at the stigma for pollen germination (Fig. 1a), at the mid style (Fig. 1b), style base (Fig. 1c), and ovary cavity for pollen tube growth (Fig. 1d). In self- and cross-pollinations of *S. stoloniferum*, a large number of pollen grains germinated (Table 3), grew

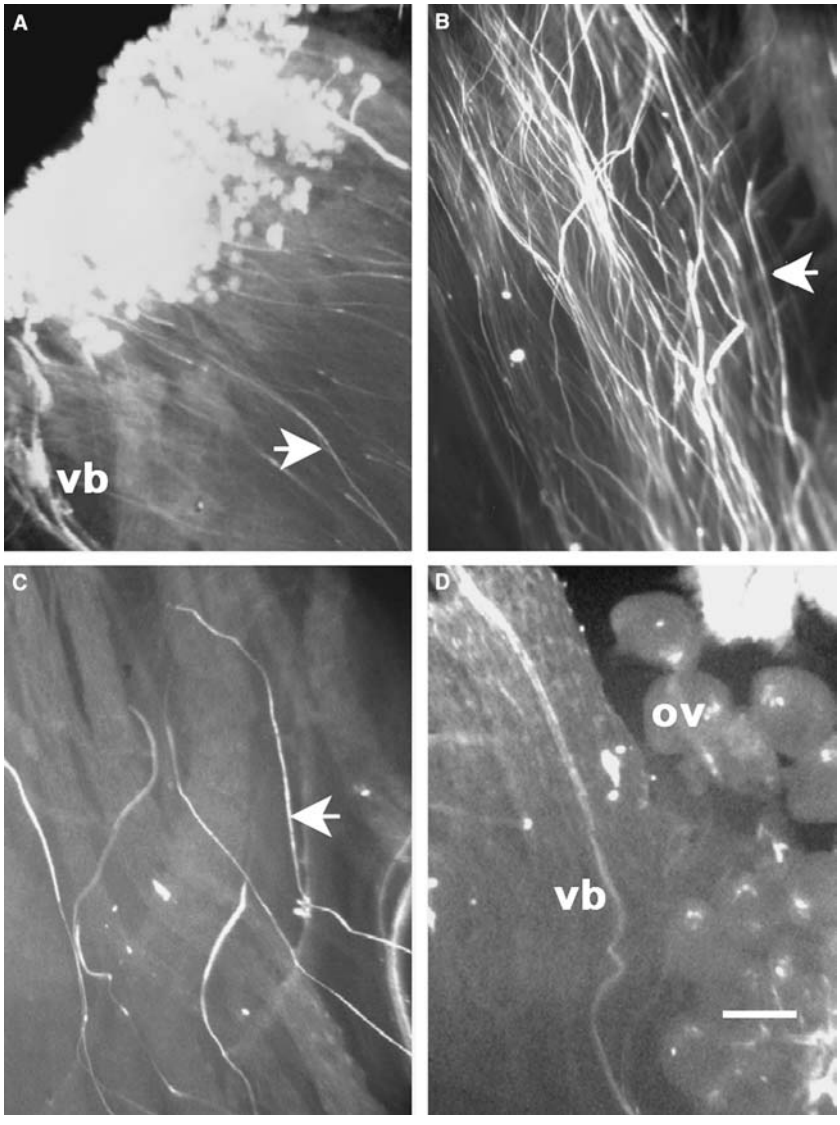

Fig. 1a–d Pollen-pistil interactions of self pollinated *Solanum stoloniferum*, *S. stoloniferum* × *Solanum cardiophyllum* and *S. stoloniferum* × *S. pinnatisectum*. **a** Stigma of *S. stoloniferum* × *S. pinnatisectum* showing many ungerminated pollen grains. **b** Pollen tubes present in the mid-style of self-pollinated *S. stoloniferum*. **c** Style base of *S. stoloniferum* × *S. cardiophyllum* showing pollen tubes. **d** Ovules of *S. stoloniferum* × *S. pinnatisectum* with no pollen tubes. *Arrows* Representative normal pollen tubes, *ov* ovule, *vb* vascular bundle. *Bar* 100 μm

Table 3 Observed pollen tube growth in styles of crosses of *S. stoloniferum* (*sto*), *S. cardiophyllum* (*cph*), *S. pinnatisectum* (*pnt*), haploid-species hybrids (*H-S*), and *S. tuberosum* haploids (*HAP*) haploids

Cross	Type[a]	Pollen germination	Average pollen tube growth	Maximum pollen tube growth	Figure[b]	Seed Formation
sto (4x) × cph (4x)	SC × SC	Reduced	One-half style length	Ovary	1c	No
sto (4x) × pnt (4x)	SC × SC	Reduced	One-half style length	Two-thirds style length	1a,d	No
sto (4x) × H-S (2x)	SC × SI	Reduced	Ovary	Ovary	2a,b	Yes
sto (4x) × sto (4x)	SC × SC	Full	Ovary	Ovary	1b	Yes
H-S/HAP (2x) × sto (4x)	SI × SC	Reduced	Ovary	Ovary	4c,d	No
H-S/HAP (2x) × pnt (4x)	SI × SC	None	Stigma	Stigma	3d	No
H-S/HAP (2x) × H-S (2x)	SI × SI	Full	Ovary	Ovary	4a	Yes
H-S/HAP (2x) self pollination	SI × SI	Few/none	One-quarter style length	One-quarter style length	4b	NA
pnt (4x) × sto (4x)	SC × SC	Few/none	One-quarter style length	One-half style length	2g,h	Aborted
pnt (4x) × H-S (2x)	SC × SI	Reduced	One-half style length	One-half style length	3a–c	No
pnt (4x) × pnt (4x)	SC × SC	Full	Ovary	Ovary	2e,f	Yes
cph (4x) self pollination	SC × SC	NA[c]	–	–	2c	No
cph (4x) × sto (4x)	SC × SC	NA	–	–	2d	No

[a]SC = Self-compatible, SI = Self-incompatible
[b]Notation referring to the manuscript figure
[c]Not applicable

through the style (Fig. 1b) and were observed in the ovary (Table 3). Reduced pollen germination was observed in *S. stoloniferum* × *S. cardiophyllum* (Table 3) and *S. stoloniferum* × *S. pinnatisectum* (Fig. 1a). However, normal pollen tubes were observed at the base of the style (Fig. 1c) and ovary cavity (Table 3) for *S. stoloniferum* × *S. cardiophyllum*. Normal pollen tubes were observed at mid style (Table 3), but not in the ovary (Fig. 1d) for *S. stoloniferum* × *S. pinnatisectum*. Many pollen grains did not germinate (Fig. 2a) in *S. stoloniferum* × H-S, but pollen tubes were commonly observed in the ovary cavity (Fig. 2b).

Crossing and pollen-pistil interaction data were not conclusive for *S. cardiophyllum* used as a female. The stigma of this genotype was abnormal in that the surface was never sticky and receptive. Therefore, little to no pollen was observed on the stigma of *S. cardiophyllum* (Fig. 2c). In one slide of *S. cardiophyllum* × *S. stoloniferum*, a single germinated pollen grain is seen. However, it did not grow further than the top one-quarter of the style, and is abnormally swollen with extensive callose deposits (Fig. 2d).

In self pollinations and inter-crosses of *S. pinnatisectum*, numerous germinated pollen grains are observed at the stigma (Fig. 2e), as well as at the style base (Fig. 2f) and ovary cavity (Table 3). *Solanum pinnatisectum* × *S. stoloniferum* crosses had a strong negative pollen-pistil interaction. Reduced pollen germination and abnormally thickened pollen tubes with accumulated callose were observed at the stigma (Fig. 2g). The maximum observed pollen tube growth in these crosses was one-half the style length (Fig. 2h). Reduced pollen germination was found in *S. pinnatisectum* × H-S crosses (Fig. 3a), and maximum pollen tube growth was observed at one-half of the style length (Fig. 3b, Table 3). These pollen tubes were abnormal, with pronounced thickening and accumulated callose. No pollen tube growth was observed in the lower part of the style and the ovary zone (Fig. 3c). A strong rejection of *S. pinnatisectum* pollen was observed on H-S/HAP stigmas, with no pollen germination and no pollen tube growth (Fig. 3d, Table 3).

Pollen tubes were observed in the ovary cavity for H-S/HAP × H-S (Table 3), although many pollen grains did not germinate in these crosses (Fig. 4a). As expected, self pollinations of H-S resulted in minimal pollen tube growth (Table 3). Consequently, no pollen tubes were observed in the ovary cavity of these crosses (Fig. 4b). For H-S/HAP × *S. stoloniferum*, pollen germination was reduced (Fig. 4c), but pollen tubes were observed in the ovary cavity (Fig. 4d). A few abnormal pollen tubes were observed in H-S/HAP × *S. stoloniferum*, but overall pollen tube morphology did not differ among H-S/HAP × H-S (Fig. 4a), *S. stoloniferum* self (Fig. 1b), *S. stoloniferum* × H-S (Fig. 2b), or H-S/HAP × *S. stoloniferum* (Fig. 4d). Unilateral differences in seed set were detected in interspecific crosses of H-S/HAP × *S. stoloniferum*, with seed being obtained when using H-S as the male only (Table 2). However, the pollen-pistil interaction was identical regardless of the direction of the cross (Table 3). Therefore, the source of the barrier occurs after the pollen tube reaches the ovary in H-S/HAP × *S. stoloniferum* crosses.

Discussion

Seed set in crosses between SI H-S/HAP and *S. stoloniferum* followed the SC rule, with success only

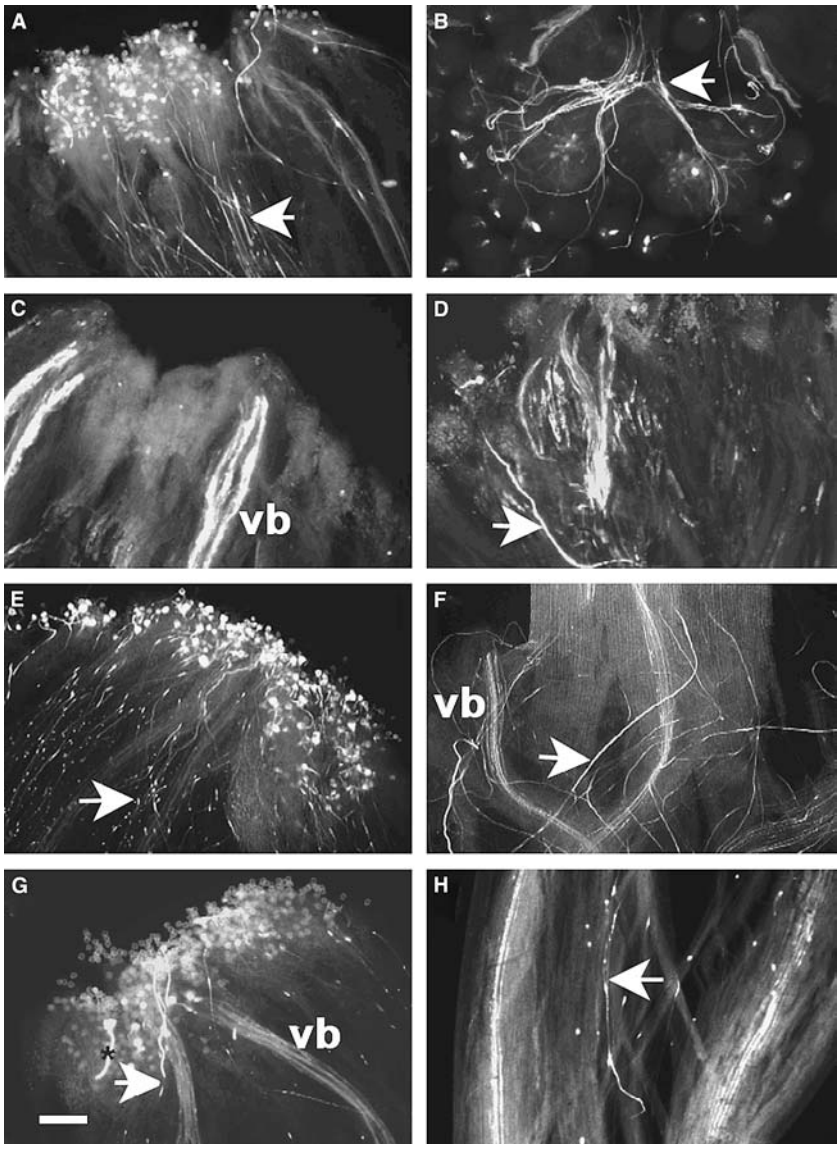

Fig. 2a–h Pollen-pistil interaction of *S. stoloniferum*, *S. cardiophyllum*, *S. pinnatisectum*, and haploid-species hybrids (H-S)/*S. tuberosum* haploids (HAP). **a** Stigma of *S. stoloniferum* × H-S with many ungerminated pollen grains. **b** Pollen tubes by ovules of *S. stoloniferum* × H-S. **c** Stigma of self-pollinated *S. cardiophyllum* with no pollen grains present. **d** Stigma of *S. cardiophyllum* × *S. stoloniferum* with one germinated pollen grain and pollen tube. **e** Stigma of self-pollinated *S. pinnatisectum* having a large number of germinated pollen grains and pollen tubes. **f** Style base of self-pollinated *S. pinnatisectum* showing many pollen tubes. **g** Stigma of *S. pinnatisectum* × *S. stoloniferum* with ungerminated pollen grains, pollen tubes showing an incompatible or abnormal reaction, and normal pollen tubes. **h** Mid-style of *S. pinnatisectum* × *S. stoloniferum*. *Arrows* Representative normal pollen tubes, *ov* ovule, * pollen tube with abnormal growth, *vb* vascular bundle. *Bar* 100 μm

occurring in SC *S. stoloniferum* × SI H-S crosses. UI due to stylar barriers has been reported in SC *S. verrucosum* × SI *S. bulbocastanum*, SC *S. verrucosum* × SI *S. tuberosum* haploids, and SC *S. pinnatisectum* × SI *S. cardiophyllum* (Abdalla and Hermsen 1972; Hermsen and Rammana 1976; Kuhl et al. 2002). In H-S/HAP-*S. stoloniferum* crosses, the reciprocal difference in seed set was not due to a stylar barrier, and is therefore unique from the UI described for other *Solanum* species. Unilateral incongruity exists when crossing barriers occur in one direction, but is not related to SI or stylar barriers (Liedl and Anderson 1993). In H-S/HAP × *S. stoloniferum* crosses, it is not clear if failure of hybridization is pre- or post-zygotic. Since progeny were recovered in *S. stoloniferum* × H-S crosses and EBNs matched for both parents, this barrier is probably not EBN related. Other pre-zygotic mechanisms not related to stylar barriers have been observed in interspecific crosses of other plant families. These include ovarian/ovule inhibition of pollen tubes or failure of male and female nuclei to fuse (Liedl and Anderson 1993). None of these mechanisms have been reported in *Solanum*, and warrant further investigation in these crosses.

In SI *S. tuberosum* haploids × SC *S. verrucosum*, S-RNases were needed for stylar inhibition of pollen tubes (Eijlander 1998). The 2x H-S/HAP parents are SI, and must have S-RNase activity. Therefore, the lack of *S. stoloniferum* pollen tube rejection in 2x H-S/HAP styles is also of interest. The mechanism of SC and the identity of the S-alleles in *S. stoloniferum* is not known, and therefore multiple possibilities exist with regard to the lack of stylar barriers. These include: (1) *S. stoloniferum* has non-functional S-alleles that result in SC and are not recognized by SI styles; (2) *S. stoloniferum* has active pollen SC factors that are unrelated to the S-locus, which suppress or do not initiate UI in SI *S. tuberosum* styles; (3) S-locus heterozygous *S. stoloniferum* pollen

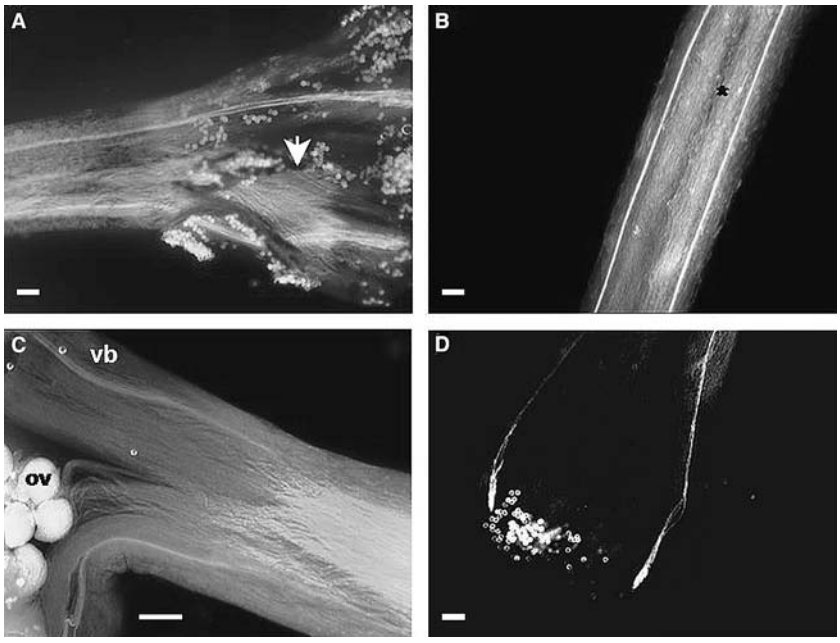

Fig. 3a–d Pollen-pistil interactions of reciprocal pollinations of *S. pinnatisectum* and H-S. **a** Stigma of *S. pinnatisectum* with germinated pollen grains and pollen tube growth of H-S. **b** Mid-style of *S. pinnatisectum* with abnormal, thickened pollen tube growth, and irregular callose depositions. **c** Style base and ovary of *S. pinnatisectum* without pollen tube growth. **d** Absence of pollen germination and no pollen tube growth on the stigma of H-S/HAP with *S. pinnatisectum* pollen. *Arrows* Representative normal pollen tubes, *ov* ovule, * pollen tube with abnormal growth, *vb* vascular bundle. *Bar* 100 μm

breaks down SI due to competitive interaction, which in turn breaks down interspecific stylar barriers.

Bilateral stylar barriers were present in *S. stoloniferum*-*S. pinnatisectum* and H-S/HAP-*S. pinnatisectum* crosses, and varying degrees of pollen rejection have been reported in interspecific crosses with 2x and 4x *S. pinnatisectum* (Novy and Hanneman 1991; Kuhl et al. 2002; Ramon and Hanneman 2002). The autotetraploid *S. pinnatisectum* used in this research is SC, as would be predicted by competitive interaction of S-alleles (Luu et al. 2001). This may break down any S-locus contribution to UI. Therefore, the persistence of stylar barriers suggests that they operate independently of SI. This was previously reported for 2x *S. pinnatisectum*-2x *S. cardiophyllum* crosses (Kuhl et al. 2002). Given this fact, the occurrence of stylar barriers in many interspecific crosses involving *S. pinnatisectum* might be species- and genotype-specific. This is not unexpected, since this species is more distantly related within section *Petota* (Spooner and Hijmans 2001). Polyploidy does not necessarily result in a complete breakdown of stylar barriers however. The presence of functional and nonfunctional alleles at the S-locus can result in reduced incompatibility both within and between SC and SI genotypes regardless of ploidy (Hauck et al. 2002). This might explain why bilateral incompatibility is maintained in

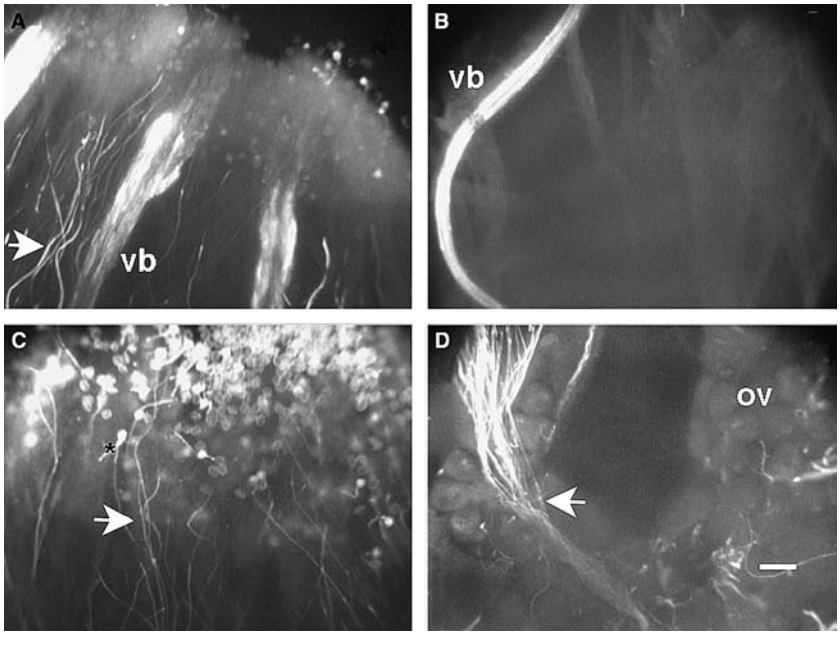

Fig. 4a–d Pollen-pistil interaction of self-pollinated H-S, H-S × H-S, and H-S × *S. stoloniferum*. **a** Stigma of H-S/HAP × H-S with germinated pollen grains and pollen tubes. **b** Style base of self-pollinated H-S with no pollen tubes. **c** Stigma of H-S × *S. stoloniferum* with germinated and ungerminated pollen grains and pollen tubes. **d** Many pollen tubes present near the ovules of H-S/HAP × *S. stoloniferum*. *Arrows* Representative normal pollen tubes, *ov* ovule, * pollen tube with abnormal growth, *vb* vascular bundle. *Bar* 100 μm

crosses between 4x SC *S. pinnatisectum* and certain SI diploids (Novy and Hanneman 1991; Ramon and Hanneman 2002; Dinu et al. 2005). The diversity of S-alleles in *S. pinnatisectum* needs to be determined to confirm this hypothesis.

Despite the observation of bilateral pollen tube inhibition, fruit was observed when *S. pinnatisectum* was used a female parent, and in crosses with *S. stoloniferum* these fruit contained aborted seeds. Similar observations were reported by Ramon and Hanneman (2002), where a single viable *S. tuberosum* × *S. pinnatisectum* progeny was obtained from crosses with a *S. pinnatisectum* accession that caused strong pollen tube inhibition in 2x *S. tuberosum* styles. This indicates that interspecific stylar barriers in *S. pinnatisectum* may be environmentally sensitive, although the specific conditions needed to break down these barriers have not been reported. Consequently, post-zygotic barriers operating independently of EBN likely prevented seed formation in 4x 2EBN *S. pinnatisectum* × 4x 2EBN *S. stoloniferum*. Ploidy manipulations, phytohormones and embryo rescue were used to create diploid and triploid bridge hybrids between 2x 2EBN *S. verrucosum* and 2x 1EBN/4x 2EBN *S. pinnatisectum* (Dinu et al. 2005). These methods may aid hybridization in the crosses reported here, creating bridge hybrids from *S. pinnatisectum* × *S. stoloniferum* crosses. Introgression of 4x 2EBN two species bridge hybrids could then proceed through several strategies involving ploidy manipulations and embryo rescue (Adiwilaga and Brown 1991; Janssen et al. 1997; Ortiz 1998).

In 4x 2EBN *S. stoloniferum* × 4x 2EBN *S. cardiophyllum*, immature fruit were formed but did not contain seeds. While we could not discriminate whether fertilization occurred in these crosses using style squashes; the occurrence of pollen tubes in the ovary and immature fruit strongly indicate fertilization followed by a subsequent post-zygotic failure of seed formation. Furthermore, the lack of aborted seeds in the immature fruit suggests that this barrier is acting early in seed development.

Unique hybridization barriers were observed with *S. stoloniferum*. These barriers may not have been present in previous bridge crosses using *S. acaule*. *S. acaule* and *S. stoloniferum* are both disomic 4x 2EBN potato species clustered in clade 4 of *Solanum* section *Petota* (Spooner and Hijimans 2001). However, each belongs to a separate series, *S. acaule* to *Acaulia* and *S. stoloniferum* to *Longipedicellata*, with *Longipedicellata* having the B genome that is unique among section *Petota* (Matsubayashi 1991). Therefore, inter-species hybridizations using *Longipedicellata* may present unique obstacles associated with the B genome. Of particular interest is the unilateral seed set in crosses with 2x H-S/HAP, which was not related to stylar inhibition of pollen tubes. Understanding the biology and genetics of SC in *S. stoloniferum* will likely be important for use of *S. stoloniferum* as a bridge species. This research is important for potato enhancement efforts. Hayes and Thill (2002) reported that *S. stoloniferum* and *S. pinnatisectum* combined high levels of late blight resistance and cold sweetening resistance in individual genotypes. *Solanum cardiophyllum* also has high levels of late blight resistance (Zlesak and Thill 2002). Development of a co-current introgression method using these species would benefit potato breeding and germplasm enhancement by expanding the genetic diversity for both traits simultaneously.

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References

Abdalla MMF, Hermsen JGTh (1972) Unilateral incompatibility: hypothesis, debate and implications for plant breeding. Euphytica 21:37–47

Adiwilaga K, Brown CR (1991) Use of 2*n* pollen-producing triploid hybrids to introduce tetraploid Mexican wild species germplasm to cultivated tetraploid potato gene pool. Theor Appl Genet 81:645–662

Bamberg JB, Hanneman RE Jr, Palta JP, Harbage JF (1994) Using disomic 4x(2EBN) potato species' germplasm via bridge species *Solanum commersonii*. Genome 37:866–870

Budin KZ, Gavrilenko TA (1994) Genetic basis of remote hybridization in potato. Rus J Genet 30:1188–1196

Cipar MS, Peloquin SJ, Hougas RW (1964) Inheritance of incompatibility in hybrids between *Solanum tuberosum* haploids and diploid species. Euphytica 13:163–172

Cruz-Garcia F, Hancock CN, McClure B (2003) S-RNase complexes and pollen rejection. J Exp Bot 54:123–130

Dinu II, Thill CA (2004) Endosperm and embryo development in interspecific incompatible *Solanum* crosses (Abstr). Am J Potato Res 81:57

Dinu II, Hayes RJ, Kynast RG, Phillips RL, Thill CA (2005) Novel inter-series hybrids in *Solanum*, section *Petota*. Theor Appl Genet 110:403–415

Eijlander R (1998) Mechanisms of self-incompatibility and unilateral incompatibility in diploid potato (*Solanum tuberosum* L.). PhD Thesis, Wageningen Agricultural University, Wageningen

Hanneman RE Jr (1989) The potato germplasm resource. Am Potato J 66:655–667

Hanneman RE Jr (1999) The reproductive biology of the potato and its implication for breeding. Potato Res 42:283–312

Hauck NR, Yamane H, Tao R, Iezzoni A (2002) Self-compatibility and incompatibility in tetraploid sweet cherry (*Prunus cerasus* L.). Sex Plant Reprod 15:39–46

Hayes RJ, Thill CA (2002) Co-current introgression of economically important traits in a potato-breeding program. Am J Potato Res 79:173–181

Helgeson JP, Pohlman JD, Austin S, Herbalach GT, Wielgus SM, Ronis D, Zambolim L, Tooley P, McGrath JM, James RV, Stevenson WR (1998) Somatic hydrids between *Solanum bulbocastanum* and potato: a new source of resistance to late blight. Theor Appl Genet 96:738–742

Hermsen JGTh (1978) Genetics of self-incompatibility in dihaploids of *Solanum tuberosum* L. 2. Detection and identification of all possible incompatibility and compatibility genotypes in six F_1s from interdihaploid crosses. Euphytica 27:13–17

Hermsen JGTh, Rammana MS (1973) Double-bridge hybrids of *Solanum bulbacastanum* and cultivars of *S. tuberosum*. Euphytica 18:27–35

Hermsen JGTh, Rammana MS (1976) Barriers to hybridization in of *Solanum bulbacastanum* Dun. and *S. verrucosum* Schlechtd. and structural hybridity in their F_1 plants. Euphytica 25:1–10

Hermsen JGTh, Taylor LM, van Breukelen EWM, Lipski A (1978) Inheritance of genetic markers from two potato dihaploids and their respective parent cultivars. Euphytica 27:681–688

Igic B, Kohn J (2001) Evolutionary relationships among self-incompatible RNases. Proc Natl Acad Sci USA 98:1316–13171

Janssen GJW, Norel A von, Verkerk-Bakker B, Janssen R, Hoogendoorn J (1997) Introgression of resistance to root-knot nematodes from wild Central American *Solanum* species into *S. tuberosum* ssp. *Tuberosum*. Theor Appl Genet 95:490–496

Johnston SA, den Nijs TPM, Peloquin SJ, Hanneman RE Jr (1980) The significance of genic balance to endosperm development in interspecific crosses. Theor Appl Genet 57:5–9

Kuhl JC, Havey MJ, Hanneman RE Jr (2002) A genetic study of unilateral incompatibility between diploid (1EBN) Mexican species *Solanum pinnatisectum* and *S. cardiophyllum* subsp. *cardiophyllum*. Sex Plant Reprod 14:305–313

Liedl BE, Anderson NO (1993) Reproductive barriers: identification, uses, and circumvention. Plant Breed Rev 11:11–154

Luu DT, Qin X, Laublin G, Yang Q, Morse D, Cappadocia M (2001) Rejection of S-heteroallelic pollen by a dual-specific S-RNase in *Solanum chacoense* predicts a multimeric SI pollen component. Genetics 159:329–335

Matsubayashi M (1991) Phylogenetic relationships in the potato and its related species. In: Tsuchiya T, Gupta PK (eds) Chromosome engineering in plants: genetics, breeding, evolution Part B. Elsevier, Amsterdam, pp 93–118

Murfett J, Strabala TJ, Zurek DM, Mou B, Beecher B, McClure BA (1996) S-RNase and interspecific pollen rejection in the genus *Nicotiana*: multiple rejection pathways pathways contribute to unilateral incompatibility between self incompatible species. Plant Cell 8:943–958

Novy RG, Hanneman RE Jr (1991) Hybridization between GP. Tuberosum haploids and 1EBN wild potato species. Am Potato J 68:151–169

Novy RG, Nasruddin A, Ragsdale DW, Radcliffe EB (2002) Genetic resistances to potato leafroll virus, potato virus Y, and green peach aphid in progeny of *Solanum etuberosum*. Am J Potato Res 79:9–18

Olsder J, Hermsen JGTh (1976) Genetics of self-compatibility in dihaploids of Solanum tuberosum L. I. Breeding behaviour of two self-compatible dihaploids. Euphytica 25:597–607

Ortiz R (1998) Potato breeding via ploidy manipulations. Plant Breed Rev 16:15–86

Ramon M, Hanneman RE Jr (2002) Introgression of resistance to late blight (*Phytophthora infestans*) from *Solanum pinnatisectum* into *S. tuberosum* using embryo rescue and double pollination. Euphytica 127:421–435

Spooner DM, Bamberg JB (1994) Potato genetic resources: sources of resistance and systematics. Am Potato J 71:325–337

Spooner DM, Hijimans RJ (2001) Potato systematics and germplasm collecting, 1989–2000. Am J Potato Res 78:237–268

Zlesak DC, Thill CA (2001) Obtaining sexual hybrids between *Solanum pinnatisectum* (1EBN) and cultivated potato germplasm. Am J Potato Res 78:489

Zlesak DC, Thill CA (2002) Variation for 2n pollen production and male fertility in wild *Solanum* germplasm resistant to *Phytophthora infestans* (Mont.) de Bary (US-8). Am J Potato Res 79:219–222